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## **Organic cation transporter 2 controls brain norepinephrine and serotonin clearance and antidepressant response**

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Running title: Role of OCT2 in monoamine clearance and antidepressant response

## **Abstract**

High-affinity transporters for norepinephrine (NE) and serotonin (5-HT), which ensure neurotransmitter clearance at the synapse, are the principal targets of widely used antidepressant drugs. Antidepressants targeting these high-affinity transporters, however, do not provide positive treatment outcomes for all patients. Other monoamine transport systems, with lower affinity, have been detected in the brain, but their role is largely unknown. Here we report that OCT2, a member of the polyspecific organic cation transporter (OCT) family, is expressed notably in the limbic system and implicated in anxiety and depression-related behaviors in the mouse. Genetic deletion of OCT2 in mice produced a significant reduction in brain tissue concentrations of NE and 5-HT and in *ex vivo* uptake of both these neurotransmitters in the presence of the dual 5-HT-NE transport blocker, venlafaxine. *In vivo* clearance of NE and 5-HT evaluated using microiontophoretic electrophysiology was diminished in the hippocampus of OCT2<sup>-/-</sup> mice in the presence of venlafaxine, thereby affecting post-synaptic neuronal activity. OCT2<sup>-/-</sup> mice displayed an altered sensitivity to acute treatments with NE and/or 5-HT-selective transport blockers in the forced-swim test. Moreover, the mutant mice were insensitive to long-term venlafaxine treatment in a more realistic, corticosterone-induced, chronic depression model. Our findings identify OCT2 as an important post-synaptic determinant of aminergic tonus and mood-related behaviors and a potential pharmacological target for mood disorders therapy.

**Keywords:** organic cation transporter; mood; depression; aminergic neurotransmission; antidepressant response

## **Introduction**

Neurotransmitter transporters exert a crucial role in the control of synaptic transmission by ensuring the rapid clearance of the released transmitters from the synaptic cleft and their recycling into the nerve endings. In monoaminergic pathways, this clearance is carried out principally by high-affinity sodium-driven transporters for dopamine (DA), 5-HT and NE, embedded in the plasma membrane of the pre-synaptic terminal. These high-affinity reuptake transporters are the primary target for various psychoactive compounds including some of the classical antidepressants, which potently inhibit the serotonin (SERT) and/or norepinephrine (NET) transporters.<sup>1,2</sup> This notion has been for decades a compelling argument in support of the “monoamine” hypothesis of depression, which assumes that hypoactivity of 5-HT and NE pathways occurs during depression that can be counterbalanced by antidepressant treatment.<sup>3</sup> Central serotonergic and noradrenergic pathways control a wide range of functions including mood and emotion, and dysfunction of these circuits has indeed been implicated in the etiology and pathophysiology of anxiety and depression.<sup>4</sup>

While the critical role of the high-affinity transporters in monoamine clearance and antidepressant action is firmly established, it has been suspected for years that additional monoamine clearance systems may exist in the brain. Low-affinity monoamine uptake through non-cognate transporters has been suggested in earlier studies using transport, voltammetry or microdialysis approaches, and selective blockers and mouse mutants for the high-affinity transporters.<sup>5-7</sup> The rigorous interpretation of these studies has been confounded, however, by the limited knowledge concerning the specificity of the inhibitors used as pharmacological tools. Thus, the physiological function of these low-affinity uptake components in CNS has remained elusive. Potential candidates that could ensure low-affinity monoamine transport in the brain include the OCT family<sup>8-10</sup> and the plasma membrane monoamine transporter (PMAT),<sup>11</sup> all of which have the capacity to transport aminergic

neurotransmitters *in vitro*. OCTs have been investigated principally for their role in the handling of organic cations in peripheral organs,<sup>12, 13</sup> and are currently emerging as novel but ill-characterized components of aminergic neurotransmission. Mice deficient for OCT3 show changes in the neural and behavioral responses to environmentally-induced variations in osmolarity, altered monoamine neurotransmission in the brain and increased sensitivity to psychostimulants,<sup>9, 14</sup> suggesting a “back-up” role in monoamine clearance, supported by studies of mice lacking SERT.<sup>15</sup> OCT3 was also shown to play an important role in MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)-induced neurodegeneration in dopaminergic pathways, by allowing the release of the neurotoxic derivative MPP<sup>+</sup> in the extracellular space.<sup>16</sup>

OCT2 is another member of the OCT family, which was detected previously in the brain.<sup>10, 17</sup> On the basis of its ability to transport 5-HT and NE, we hypothesized that this transporter could modulate monoaminergic neurotransmission in complement to the high-affinity transporters, and play a role in the control of mood and related functions. To test this possibility, we investigated the contribution of OCT2 to monoamine clearance *in vivo* in the brain, and its functional consequences on post-synaptic neuronal activity, as well as on anxiety and depression-related behaviors and the response to antidepressants.

## **Material and methods**

### *Animals*

OCT2<sup>-/-</sup> mice were previously generated by homologous recombination.<sup>13</sup> Heterozygous animals with ten backcross generations into C57BL6/J were bred to generate wild-type and knockout littermates, which were genotyped as described previously<sup>13</sup> and 8–12 weeks old mice were used for the experiments. Animal care and experiments were conducted in accordance with standard ethical guidelines (European Communities Council Directive for the Care and Use of Laboratory Animals, 86/609/EEC) and approved by local ethical committees.

### *Immunohistochemistry and immunoautoradiography*

Free-floating immunohistochemistry was performed as detailed in Supplementary Material and methods. For immunoautoradiography, kidney or brain cryosections were fixed with 4% paraformaldehyde/PBS and incubated successively with OCT2 (1/500), OCT3 (1/500),<sup>9</sup> or PMAT antibodies raised against peptide sequence YLHHKYPGTSIVF (1/500; Agro-Bio), and with [<sup>125</sup>I] anti-rabbit IgGs (Perkin Elmer, Boston, MA, USA) and exposed to film for 3–8 days. Analysis and quantification of autoradiograms were carried out with MCID software (Imaging Research, Brock University, St. Catherine's, Ontario, Canada).

### *Monoamine uptake*

Mice were killed by decapitation and the hippocampus and frontal cortex were dissected, minced on ice and resuspended in ice-cold sucrose (0.32 M). The cells were dissociated by filtering through nylon meshes of decreasing pore size (180 to 60 μm, Small Parts, Miramar, FL, USA) and resuspended in ice-cold sucrose. The cellular suspension was preincubated 10 min at 37 °C in 3 vol of Krebs Ringer HEPES (KRH) LiCl buffer (25 mM HEPES–KOH, pH

7.4, 125 mM LiCl, 4.8 mM KCl, 5.6 mM D(+)-glucose, 1.2 mM CaCl<sub>2</sub>, 1.2 mM KH<sub>2</sub>PO<sub>4</sub> and 1.2 mM MgSO<sub>4</sub>) in the presence of 5 μM venlafaxine (Tocris Bioscience, Bristol, UK), 100 μM GBR12935, 10 μM pargyline and 10 μM reserpine (Sigma-Aldrich, St Louis, MO, USA), and incubated for 15 min at 37 °C in the same buffer supplemented with 10 μM [<sup>3</sup>H] NE, [<sup>3</sup>H] 5-HT, [<sup>3</sup>H] DA or [<sup>3</sup>H] glutamate (Perkin Elmer). The effect of citalopram on endogenous OCT2 activity was evaluated in the presence of increasing concentrations of this antidepressant (0.1–10 mM) during pre-incubation and uptake. The reaction was terminated by rapid filtration through Unifilter-96 GF/C filters (Perkin Elmer) and after washing the radioactivity retained on the filters was assessed by liquid scintillation. The protein concentration in tissue extracts was measured by the method of Bradford. OCT-mediated uptake was quantified by inhibition with the specific inhibitor decynium 22 (1,1'-diethyl-2,2'-cyanine iodide)<sup>18</sup> (500 μM; Sigma-Aldrich). Data are expressed as mean ± s.e.m. of D22-sensitive uptake for 4–6 independent experiments performed in triplicate.

#### *Determination of monoamine brain content*

Brain structures were assessed for monoamine and metabolite content by HPLC as previously reported.<sup>19</sup>

#### *Iontophoretic electrophysiology*

Extracellular recordings of pyramidal neurons in the CA3 region of the hippocampus were carried out using multi-barreled glass micropipettes as described in Supplementary Material and methods.<sup>20</sup> In agreement with its ability to block both NE and 5-HT reuptake in mice,<sup>21</sup> venlafaxine at the dose of 16 mg per kg, subcutaneous, but not 8 mg per kg, produced a reduction of the firing rate of CA3 pyramidal neurons in wild-type and OCT2<sup>-/-</sup> mice. The excitatory current of quisqualate was thus increased for both groups to maintain a firing

activity rate similar to that before venlafaxine injection at the dose of 8 mg per kg. The time required for the firing activity to recover 50% of the initial firing rate after microiontophoretic application ( $RT_{50}$  value) was used as an index of the capacity of neurons of the dorsal hippocampus to remove NE or 5-HT from the synaptic cleft in the presence or absence of venlafaxine (8 and 16 mg per kg).<sup>21</sup>

#### *Receptor and transporter autoradiography*

Male mice were killed by cervical dislocation and brains were rapidly extracted and frozen on dry ice. For receptor and transporter binding, cryosections were processed as previously reported with [<sup>3</sup>H] nisoxetine for the NET,<sup>22</sup> [<sup>3</sup>H] citalopram for the SERT<sup>23</sup> and [<sup>3</sup>H] WAY100635 for the 5-HT<sub>1A</sub> receptor<sup>24</sup> (Perkin Elmer). Quantification of autoradiograms were carried out with MCID software (Imaging Research).

#### *Behavioral and pharmacological studies*

Most behavioral studies were performed during the inactive phase (09:00–13:00) with aged-matched (8–10 weeks), naive mice. Each group was composed of balanced numbers of both genders and similar effects of genotype were observed for male and female mice.

General locomotor activity was assessed in activity chambers (25 X 15 X 20 cm) placed in a rack containing four infrared photobeams located 15 mm above the floor along the long and short axis. Photobeam interruptions were recorded during 2 h with a six-channel amplifier connected to a computer (Imetronic, Bordeaux, France). Motor coordination was evaluated with a rotating rod apparatus (Bioseb, Chaville, France) with speed increased linearly from 4 to 40 r.p.m. during a 5 min observation period. Mice were given five trials separated by 30 min intervals and scored for their latency to fall off the rotarod.



The open-field consisted of a white Plexiglas field (100 x 100 x 30 cm) with the center brightly illuminated (2 500 lux). General locomotor activity in the center and periphery of the open-field were scored for 9 min. The time and number of entries in the center zone (60 x 60 cm) were evaluated as an index of anxiety-related response.

The elevated O-Maze consisted of an annular runway positioned 40 cm above the floor and divided into two opposing 90 ° closed sectors and two 90 ° open sectors. The mice were individually placed in the closed sector and their behavior recorded over a 5-min period. The time spent in each sector and the number of sector entries (a sector entry was defined as all four of the paws being placed in a sector) were determined by video tracking (Viewpoint, Lyon, France).

Novelty-suppressed feeding was performed as previously described.<sup>25</sup> The mice were food-restricted for 24 h with water *ad libitum*, and the test performed in a brightly lit (800–900 lux) square arena (40 x 40 x 20 cm) with a standard food pellet (6 g) at the center. The latency to feed was assessed and the animals immediately transferred to their home cage, where food consumption was measured for 10 min. The animals were weighed before and after food deprivation. Food consumption in the home cage and percentage of weight lost after food deprivation were used as relative controls for animal hunger.

Immobility was evaluated in the forced swimming and the tail suspension tests. In the forced-swim test, the mice were placed individually in a glass beaker filled with 25 ± 1 °C water to a depth of 12 cm.<sup>26</sup> The duration of immobility (i.e. the time during which the mice make only minimal movements to stay afloat) was recorded during the last 4 min of the 6-min testing period, after 2 min of habituation. In the tail suspension test, mice were suspended in the test apparatus and immobility time was automatically recorded during a 6-min test session (Bio-TS, Bioseb). Venlafaxine (Tocris Bioscience), reboxetine (Pfizer, Groton, CT, USA) and

citalopram (Lunbeck, Copenhagen, Denmark) were dissolved in saline (0.9 % NaCl [wt/vol]) and administered intraperitoneally in a volume of 10 ml per kg 30 min before forced-swim.

For the sucrose preference test, singled-house mice were first habituated for 48 h to drink water from two bottles. On the following three days, the mice could choose between a water bottle and a 1% (wt/vol) sucrose solution bottle, switched daily. Sucrose solution intake for 24 h was measured during the last two days and expressed as a percentage of the total amount of liquid ingested .

The splash test was carried out in home cage and consisted in spraying 200  $\mu$ l of a 10% (wt/vol) sucrose solution on the mouse's snout using a small atomizer. The grooming frequency and latency were then recorded for 5 min.

The coat state was evaluated as the sum of the score of different parts of the body, ranging between 0 for a well-groomed coat and 2 for an unkempt coat for head, neck, dorsal/ventral coat, and between 0 and 1 for tail and forepaws/hindpaws.

The social interaction test was performed in a white plastic arena (42 x 42 cm) containing an empty wire mesh cage (10 x 6.5 cm) at an extremity of the field and monitored by video tracking (Viewpoint). The test male mouse was allowed to explore the arena for two consecutive 2.5 min sessions. During the second session, an unfamiliar male mouse that had no prior contact with the subject mice was placed in the cage. The time spent by the test mouse in the interaction zone, i.e., a 8-cm wide region surrounding the mesh cage, and the opposite corners of the arena, was measured in both sessions.

To induce a chronic depression-like state, individually-housed male mice were administered corticosterone (35  $\mu$ g per ml; Sigma-Aldrich) dissolved in 0.45% (wt/vol) hydroxypropyl- $\beta$ -cyclodextrin (Sigma-Aldrich) or vehicle (0.45% hydroxypropyl- $\beta$ -cyclodextrin) in drinking water during seven weeks.<sup>27</sup> After four weeks, the mice were tested during the light phase successively for coat state, sucrose consumption, social interaction,

elevated O-maze, splash test and novelty-suppressed feeding. Venlafaxine was administered daily (16 mg per kg per day, intraperitoneally, at 5–7 p.m.) during the last three weeks of the corticosterone regimen and the mice tested again. Plasma corticosterone concentrations were determined using radioimmune assay kits (MP Biomedicals, Solon, OH, USA) and expressed as  $\text{ng}\cdot\text{ml}^{-1}$  of plasma.

### *Statistics*

PRISM (GraphPad Software, San Diego, CA, USA) and Statview 5.0 (SAS Institute Inc., Cary, NC, USA) were used for statistical calculations. Student's *t*-test was used for comparisons of mean values for the two genotypes in the behavioral experiments, neurotransmitter uptake, ligand binding and immunautoradiography experiments and HPLC dosages. For microiontophoretic electrophysiology and behavioral tests, data were analyzed using two-way analysis of variance (ANOVA) (treatment x genotype), followed by Fisher's Protected LSD test. The Mantel-Cox test was used to compare latencies to feed in the novelty-suppressed feeding test. Statistical significance was set at  $P < 0.05$ .

## **Results**

### *OCT2 is enriched in the limbic system*

The anatomical distribution of OCT2 was investigated by immunohistochemistry on mouse brain sections with affinity-purified antibodies raised against a synthetic peptide corresponding to an epitope of the rodent protein diverging from other OCT subtypes. Expression of OCT2 was widespread, but notably enriched in specific areas. Specifically, strong OCT2 labeling was found in the frontal cortex, shown here for primary and secondary motor and cingulate cortices (Figure 1a), in CA2, CA3 and CA1 of the hippocampus (Figure 1b) and in the amygdala (Figure 1c). In contrast, OCT2 was only detected in some aminergic

neuron-containing regions, such as the locus coeruleus (Figure 1d) and dorsal raphe (Figure 1e) but not in the substantia nigra or ventral tegmental area (not shown). In the locus coeruleus, OCT2 was found in most but not all noradrenergic neurons, labeled by tyrosine hydroxylase antibodies (Figure 1d). In the dorsal raphe, OCT2 was weakly expressed in a fraction of serotonergic neurons, labeled by tryptophan hydroxylase antibodies (Figure 1e). Co-labeling experiments with antibodies directed against the neuronal marker NeuN confirmed the neuronal expression of OCT2 in all brain regions (Figure 1f-h). No obvious expression of OCT2 was detected in astrocytes (Figure 1i). In OCT2<sup>-/-</sup> mice, the labeling was abolished in kidney as well as in all brain structures, demonstrating the specificity of the antibody (Supplementary Figure 1 and Figure 1a-c). Altogether, these experiments indicate that OCT2 is markedly expressed in the limbic regions of mouse brain.

*OCT2 controls anxiety-related behavior and immobility in the forced-swim and tail suspension tests*

The limbic and prefrontal cortical regions, which express the highest levels of OCT2, play an important role in the response to stress or threats<sup>28</sup> and in mood disorders.<sup>4, 29, 30</sup> We thus carried out a set of mood-related behavioral tests to assess the level of anxiety (open-field, elevated O-maze, novelty-suppressed feeding) and so-called behavioral despair (forced-swim and tail suspension tests) of OCT2<sup>-/-</sup> mice. OCT2<sup>-/-</sup> mice characterized previously are normally viable and fertile, and standard histological analysis did not show evident morphological anomalies.<sup>13</sup> General locomotor activity of the mutants evaluated in an actimeter chamber was similar to that of wild-types, while motor coordination in a rotarod test was slightly impaired (Supplementary Figure 2). In the open-field apparatus, which evaluates locomotor activity in a novel environment as well as the level of anxiety, activity at the periphery of the open-field was comparable for the two genotypes (Figure 2a), confirming the

observations in the actimeter chamber. In this test, however, the time as well as the horizontal and rearing activity in the center of the open-field were significantly increased in the OCT2<sup>-/-</sup> mice (Figure 2a), indicating a decreased level of anxiety. In the elevated O-maze, OCT2<sup>-/-</sup> mice showed an increase in the time spent in the open zone as well as decreased latency to enter this zone (Figure 2b). In a third paradigm, novelty-suppressed feeding, which evaluates the latency of the animal to consume food in a novel aversive environment, OCT2<sup>-/-</sup> mice exhibited a decreased latency to feed in comparison to wild-type mice, whereas body weight loss and food consumption in the home cage were similar in both groups (Figure 2c). These experiments show that OCT2 deficiency in mice results in decreased anxiety level, as evaluated by these three conflict paradigms. Next, we assessed immobility during exposure to inescapable stress using the forced-swim and the tail suspension tests, two tests classically used to evaluate the efficacy of antidepressants. In these two paradigms OCT2<sup>-/-</sup> mice showed a marked increase in immobility time compared to wild-type mice, indicative of an increased level of resignation (Figure 2d,e). Thus, consistent with its predominant expression in limbic regions, OCT2 is implicated in the control of mood-related behaviors like anxiety and acute “behavioral despair” in antidepressant-related tests.

#### *OCT2 participates in 5-HT and NE clearance in aminergic projection regions*

Based on OCT2 properties established *in vitro*,<sup>31</sup> we speculated that this transporter could participate in monoamine uptake in the limbic system, broadly innervated by monoaminergic fibers. To examine the transport properties of endogenous OCT2, we measured monoamine uptake *ex vivo* in fresh cell suspensions from two regions with high OCT2 expression, hippocampus and cortex, from wild-type and OCT2<sup>-/-</sup> mice (Figure 3a). Uptake of tritiated NE, 5-HT and DA was carried out in the presence of high-affinity monoamine transporter inhibitors and sodium-depleted buffer, in order to minimize transport by high-affinity

systems. The contribution of OCT-mediated uptake to total uptake, evaluated with the selective OCT inhibitor decynium 22 (D22),<sup>18</sup> was compared between wild-type and mutant mice. These experiments showed D22-sensitive uptake of the three monoamines in extracts from wild-type mice hippocampus ( $8.44 \pm 0.62 \text{ fmol.mg}^{-1}.\text{min}^{-1}$  for NE,  $1.82 \pm 0.18 \text{ fmol.mg}^{-1}.\text{min}^{-1}$  for 5-HT and  $0.62 \pm 0.11 \text{ fmol.mg}^{-1}.\text{min}^{-1}$  for DA) and cortex ( $1.00 \pm 0.10 \text{ fmol.mg}^{-1}.\text{min}^{-1}$  for NE,  $1.05 \pm 0.06 \text{ fmol.mg}^{-1}.\text{min}^{-1}$  for 5-HT and  $2.15 \pm 0.40 \text{ fmol.mg}^{-1}.\text{min}^{-1}$  for DA). Comparison of D22-sensitive uptake in extracts from the two genotypes showed a significant decrease in transport activity in OCT2<sup>-/-</sup> mice compared to wild-types for NE and 5-HT in both hippocampus and cortex, whereas DA uptake was not significantly different between genotypes (Figure 3a) nor that of another neurotransmitter not transported by OCT2, glutamate (not shown).

Tissular levels of NE, 5-HT and DA were evaluated by HPLC in wild-type and OCT2<sup>-/-</sup> mice. OCT2 deletion was associated with significant reductions in the content in NE and 5-HT in several brain regions (Figure 3b). The most conspicuous difference between genotypes was a decrease in intra-tissular level of NE in the cortex and hypothalamus (38% and 22%, respectively); significant differences were also found in the striatum, hippocampus, brain stem and cerebellum (Figure 3b). Similarly, a decrease in the intra-tissular level of 5-HT was detected in the striatum (20%), hippocampus (19%) and hypothalamus (27%) (Figure 3b). No significant differences were found for endogenous DA levels (not shown), in agreement with the results of *ex vivo* DA uptake.

The above experiments suggest that OCT2 participates in NE and 5-HT clearance in aminergic projection regions of the brain. To explore the functional consequences of OCT2 deletion on 5-HT and NE clearance, the activity of post-synaptic neurons was investigated *in vivo* by a combined microiontophoretic and electrophysiological approach in the hippocampus CA3, one of the regions with the highest OCT2 density. As previously

reported,<sup>32</sup> microiontophoretic applications of 5-HT and NE resulted in the suppression of the firing activity of CA3 pyramidal neurons. In both wild-type and OCT2<sup>-/-</sup> mice, this firing gradually recovered upon the cessation of the application of monoamines (Figure 3c, top). The recovery time for pyramidal neuron firing, before and after acute systemic administration of venlafaxine (8 or 16 mg per kg), was expressed as RT<sub>50</sub> values (Figure 3c, bottom). In basal conditions, RT<sub>50</sub> values for NE or 5-HT did not differ between wild-type and OCT2<sup>-/-</sup> mice (Figure 3c, bottom). These RT<sub>50</sub> values were significantly increased in both wild-type and OCT2<sup>-/-</sup> mice after the administration of the dual 5-HT-NE transport inhibitor venlafaxine at 16 mg per kg (Figure 3c). However, in contrast to basal conditions, RT<sub>50</sub> values after venlafaxine at doses of both 8 and 16 mg per kg were significantly higher in OCT2<sup>-/-</sup> than in wild-type mice (Figure 3c). These observations indicate that OCT2 can promote the recovery of the firing activity of CA3 hippocampal neurons after NE and 5-HT application *in vivo*, in particular when the NET and SERT are pharmacologically inactivated. Thus, OCT2 appears to play a key role in the modulation of post-synaptic neuronal activity in response to the antidepressant venlafaxine.

To rule out that the differences in uptake and clearance detected in OCT2<sup>-/-</sup> mice could be due to adaptative changes in the expression of other high or low-affinity transporters in the brain, the level of NET, SERT, OCT3 and PMAT were quantified on brain sections of mutants and wild-type mice by binding of specific radiolabeled ligands or immunautoradiography. These experiments showed that in most brain regions these transporters were expressed at a comparable level in OCT2<sup>-/-</sup> and wild-type mice (Figure 3d and Supplementary Tables 1 and 2). NET and PMAT levels were similar in most brain regions (Figure 3d and Supplementary Table 1). A moderate increase in SERT density was found in the hippocampus (8% and 14% respectively for CA3 and dentate gyrus) and cingulate cortex (25%), while OCT3 level was increased of 25% in the hippocampus CA3

(Figure 3d and Supplementary Table 2). No decrease in the level of these transporters was detected, confirming that the differences in D22-sensitive NE and 5-HT uptake and *in vivo* clearance between wild-type and OCT2<sup>-/-</sup> mice reflected indeed lack of OCT2 activity.

Finally, to better identify the pre- and/or post-synaptic sites of OCT2-mediated uptake, we explored by immunohistochemistry the fine subcellular localization of OCT2 in aminergic projection regions. In all neurons, OCT2-specific labeling was circumscribed to the soma and plasma membrane in the cell body and dendrites, as shown by MAP2 co-labeling in the cortex, hippocampus and amygdala (Figure 3e). OCT2 was not detected in pre-synaptic nerve endings labeled with antibodies against the synaptic vesicle protein synaptophysin, shown here in the cingulate cortex and hippocampus CA3 (Figure 3f). OCT2 appears thus strategically located for NE and 5-HT clearance at the plasma membrane of the dendrites and body of neurons in projection areas. Collectively, this set of experiments strongly suggests that OCT2 controls 5-HT and NE clearance at the post-synaptic level in limbic regions of the brain and can modulate neuronal activity in these regions *in vivo* in presence of the antidepressant venlafaxine.

#### *OCT2 modulates the sensitivity to antidepressants in the forced-swim test*

To determine whether OCT2 is implicated in the action of antidepressants, the behavioral response to these drugs were investigated in OCT2<sup>-/-</sup> mice using the forced-swim paradigm. In comparison to wild-type mice, OCT2<sup>-/-</sup> mice showed altered sensitivity to three antidepressants: the dual 5-HT/NE reuptake inhibitor venlafaxine, and the NET- and SERT-selective reuptake inhibitors, reboxetine and citalopram, respectively (Figure 4a-c). When treated with low doses of antidepressants, otherwise inactive in wild-type animals, OCT2<sup>-/-</sup> mice showed a marked sensitivity to the immobility-reducing effects of all three compounds (Figure 4a-c). At increasing doses of venlafaxine, the mutants showed a progressive reduction



of immobility time similar to that found in wild-type animals (Figure 4a). A similar progressive reduction of immobility time was also induced by increasing doses of reboxetine, but at the dose of 20 mg/kg this reduction was markedly stronger in the mutants than in wild-type animals, demonstrating increased sensitivity (Figure 4b). In contrast, medium to high doses of citalopram did not elicit a further reduction in immobility in the mutants as in normal mice, indicating decreased sensitivity at these higher doses (Figure 4c). To investigate whether this decreased sensitivity could be a consequence of a direct interaction of citalopram with OCT2, [<sup>3</sup>H] 5-HT uptake in cell suspensions from wild-type and mutant mice cortex were carried out in the presence of increasing concentrations of citalopram (Figure 4d). These experiments showed that citalopram had no significant action upon OCT2-mediated uptake *ex vivo*. Thus, to further characterize the modifications in the serotonergic systems that could underlie the pharmacological phenotype of OCT2<sup>-/-</sup> mice, the levels of 5-HT<sub>1A</sub> receptors were evaluated by [<sup>3</sup>H] WAY100635 binding on sections of mutants and wild-type mice brain. 5-HT<sub>1A</sub> receptor density throughout the brain showed a low but significant decrease in the cingulate cortex (14%), lateral septal nucleus (14%), and CA1 of hippocampus (8%) of mutant mice (Supplementary Table 3), confirming that subtle alterations occur in serotonergic neurotransmission pathways. Taken together, these results reveal that OCT2 is an important modulator of the response to NE and 5-HT-selective antidepressants in the FST.

*OCT2 is required for the response to long-term venlafaxine treatment in a corticosterone-induced depression model*

Long-term administration of corticosterone can induce in rodents a panel of behavioral anomalies that mimic diverse aspects of human depression and can be improved by long-term antidepressant treatment.<sup>27, 33</sup> To investigate the pathophysiological relevance of our findings to human depression, we examined whether OCT2<sup>-/-</sup> mice showed alterations in the response

to long-term venlafaxine treatment using this chronic corticosterone paradigm, which also presents the advantage of leveling out and overriding uncontrollable variations in corticosterone secretion over time (Figure 5a). Mutant and wild-type mice were evaluated in basal conditions and following chronic corticosterone administration with a battery of tests known to recapitulate diverse anomalies observed in depression. Sucrose solution consumption was used to monitor the progression of anhedonia. The elevated O-maze and the novelty-suppressed feeding test are conflict paradigms reflecting anxiety level, whereas grooming behavior in the splash test and coat state are indicators of the state of behavioral despair of the animals. After a 4 week corticosterone exposure (5 mg per kg per day), OCT2<sup>-/-</sup> and wild-type mice showed enhanced and comparable levels of circulating corticosterone (117± 13 ng.ml<sup>-1</sup> and 111± 17 ng.ml<sup>-1</sup>, respectively) and robust alterations indicative of a depressed-like state in sucrose consumption, elevated O-maze, splash test performance and coat state (Figure 5b), as well as in social interaction and novelty-suppressed feeding (Supplementary Figure 3). To determine the sensitivity to chronic administration of antidepressants, corticosterone-treated OCT2<sup>-/-</sup> and wild-type mice were again tested after a 21-days treatment with 16 mg per kg per day venlafaxine. Long-term venlafaxine reversed the effects of chronic corticosterone in wild-type mice in sucrose consumption, elevated O-maze, splash test performance and coat state, but failed to improve the performances of OCT2<sup>-/-</sup> mice (Figure 5c). No effect of venlafaxine was observed on both genotypes in the other two tests, social interaction and novelty-suppressed feeding (not shown). Indeed, although OCT2<sup>-/-</sup> mice were as sensitive as wild-type mice to the effects of chronic corticosterone exposure on the development of an overall depressed-like phenotype, they failed to develop, in contrast to wild-type mice, an improvement of this depressed phenotype upon long-term venlafaxine treatment. OCT2 appears thus to be required for the full long-term effects of venlafaxine in a chronic corticosterone-induced depression model.

## Discussion

Until recently, the high-affinity reuptake transporters were considered the principal elements responsible for the clearance of monoamines at nerve endings. The body of neurochemical and electrophysiological evidence presented herein demonstrates that a low-affinity, high-capacity transporter, OCT2, is an important actor in NE and 5-HT clearance *in vivo* in the limbic brain. *Ex vivo* transport experiments and *in vivo* combined microiontophoresis and electrophysiology indicated that OCT2-mediated clearance, although subtle, is an intrinsic component of the recovery of 5-HT and NE-suppressed firing activity of post-synaptic neurons in a monoaminergic projection region, the hippocampus CA3, once the high affinity transporters are inhibited. Such a low-affinity 5-HT clearance component was previously detected by high-speed chronoamperometry in the hippocampus of rats after SERT blockade<sup>7</sup> and of SERT mutant mice, and shown to be sensitive to the OCT-inhibitor D22.<sup>15</sup>

Clearance via OCT2 could have important consequences on NE and/or 5-HT signaling in conditions of monoamine spill-over in the presence of antidepressants but also in areas of lesser high-affinity transporter density, at distance from the aminergic varicosities. Consistently, OCT2 was found in strategic post-synaptic locations to ensure this dual function, i.e., at the plasma membrane and in the somato-dendritic compartments of neurons in regions of aminergic projections in the brain. Paracrine, or "volume" transmission, is a well-established mode of transmission for monoamines, which varies depending on brain region. Diffusion of monoamines to sites remote from the nerve end terminals has been demonstrated by cyclic voltammetry for 5-HT in the dorsal raphe and substantia nigra reticulata,<sup>34</sup> for catecholamines in mouse prefrontal cortex<sup>35</sup> and for NE in the upper layers of cingulate and sensory cortices, where it may travel up to 100  $\mu\text{m}$ <sup>36</sup> and thus be amenable to uptake by OCT2. The participation of OCT2 in physiological NE and 5-HT uptake in basal

conditions is sustained by the decreased intratissular concentrations of these neurotransmitters in aminergic projection regions in OCT2<sup>-/-</sup> mice in the absence of any pharmacological treatment. Although less pronounced, this observation is reminiscent of the reduction in monoamine content found in the brain of SERT and NET-deficient mice.<sup>37, 38</sup>

Limbic and prefrontal cortical regions are known to modulate the autonomic and endocrine responses to stress or threats<sup>28</sup> and mediate cognitive and emotional aspects of mood disorders.<sup>4, 29, 30</sup> Specific anatomical<sup>30, 39</sup> and functional<sup>30, 40, 41</sup> anomalies of these systems have been detected in patients with major depression or bipolar disorder. Alterations of anxiety and immobility in the TST and FST in OCT2<sup>-/-</sup> mice could thus be the consequence of impaired 5-HT and NE clearance in these limbic regions, all extensively innervated by serotonergic and noradrenergic fibers, although the precise mechanisms implicated are still to be determined. In addition to these areas, regions expressing lower levels of OCT2, such as the locus coeruleus and the dorsal raphe, also implicated in mood-related behavior and the response to antidepressants,<sup>42</sup> could play a part in the overall phenotype of the mutants. Although less likely, it cannot be totally excluded that other structures weakly expressing OCT2, such as microvessels,<sup>43</sup> might be implicated as well. Following acute stress including inescapable stress like forced swim, specific serotonergic and noradrenergic pathways are activated within minutes to trigger the activation of the hypothalamic-pituitary-adrenocortical axis, through direct and indirect connections to the paraventricular nucleus and peri-paraventricular nucleus regions.<sup>44-47</sup> OCT2 deletion could thus potentiate the effect of 5-HT and NE release during this activation in raphe and projection areas like hippocampus, amygdala and prefrontal cortex.<sup>48-50</sup> Furthermore, selective activation of post-synaptic 5-HT<sub>1A</sub> receptors has been shown to attenuate behavioral despair<sup>51, 52</sup> and promote anxiety,<sup>53</sup> suggesting that the decrease in 5HT<sub>1A</sub> receptor density in projection regions in OCT2<sup>-/-</sup> mice could contribute to accentuate their overall

phenotype. Similar adaptive modifications in the expression or activity of 5-HT<sub>1A</sub> receptors have been observed after long-term increase in extracellular 5-HT<sup>54</sup> and SERT deficiency.<sup>19,55</sup>

Our study disclosed that OCT2 is a critical element for the behavioral response to antidepressants in both acute and long-term paradigms. In the forced-swim test, the genetic inactivation of OCT2 potentiated the antidepressant-like effects of pharmacological blockade of the high affinity transporters by selective serotonin (SSRIs), norepinephrine (NRIs) and dual-selective (SNRIs) reuptake inhibitors. These reuptake inhibitors are believed to exert their actions mainly by elevating synaptic and/or extrasynaptic 5-HT and NE,<sup>21</sup> a concept that has been decisive to implicate serotonergic and noradrenergic mechanisms in the pathophysiology of depressive disorders. The hypersensitivity of OCT2<sup>-/-</sup> mice to NE and 5-HT uptake inhibitors at doses inactive in wild-type animals fits well with a simple scheme where the clearance of both 5-HT and NE neurotransmitters is diminished in the mutants due to the absence of OCT2, thereby intensifying the increase in synaptic NE and 5-HT concentrations provoked by the antidepressants. As we showed here, this effect was not due to decreased NET or PMAT expression which was found unaltered, nor SERT or OCT3 expression, which appeared rather increased in the mutants. In addition, OCT2<sup>-/-</sup> mice showed a relative insensitivity to higher doses of citalopram. Since several antidepressants including citalopram have been shown to inhibit OCTs in heterologous systems,<sup>56</sup> we investigated whether this decreased sensitivity could be a consequence of a direct inhibition of OCT2 by this antidepressant. Citalopram had no significant action upon D22-sensitive OCT2-specific uptake *ex vivo*, arguing against a major inhibitory effect on transport through direct interaction with OCT2. Acutely, 5-HT and dual-selective reuptake inhibitors provoke the inhibition of serotonergic neurons firing through the local activation of the 5-HT<sub>1A</sub> autoreceptors of the raphe. This mechanism, believed to dampen their effect on 5-HT extracellular levels in projection areas,<sup>57</sup> is enhanced at high concentrations of citalopram.<sup>58</sup>

At high doses of citalopram, impaired 5-HT clearance in raphe of OCT2<sup>-/-</sup> mice could thus displace this equilibrium in favor of feedback inhibition. Alternatively, decreased activity at post-synaptic 5-HT<sub>1A</sub> receptors, shown to mediate the anti-immobility effects of antidepressants in the forced-swim paradigm<sup>52, 59</sup> could also participate in this effect.

Acute “behavioral despair” tests such as the forced-swim test, although useful to evaluate antidepressant efficacy, fall short of recapitulating the complex spectrum of cognitive and emotional dysfunctions found in depressive disorders. Chronic stress and more recently chronic corticosterone exposure have been used to develop more realistic models in rodents, encompassing distinct facets of depression such as anhedonia, anxiety, social aversion and resignation.<sup>33, 60</sup> Importantly, the persistent behavioral anomalies induced in these models can be improved by long-term, but not acute, antidepressant treatment,<sup>33, 60</sup> a characteristic shared with human depression. Thus, to gain more insight into the role of OCT2 in depression and antidepressant response, we induced a general depression-like phenotype by chronic corticosterone exposure. OCT2<sup>-/-</sup> mice developed as efficiently as wild-type mice a depressed-like phenotype after chronic corticosterone exposure. In wild-type mice, long-term venlafaxine treatment reversed the depression-like anomalies induced by chronic corticosterone, an effect that had been previously reported for fluoxetine and imipramine.<sup>33, 60</sup> In contrast, the overall depressed-like phenotype of OCT2<sup>-/-</sup> mice after corticosterone exposure was insensitive to venlafaxine treatment, indicating that OCT2 is required for the full long-term effect of this antidepressant. This observation, radically contrasting with the hypersensitivity observed in the forced-swim test, can be explained by the fact that antidepressants take several weeks to exert their therapeutic actions in man as well as in animal models, and trigger mechanisms that differ, at least in part, from those implicated in their short-term action. The complex alterations that underlie their emotional and behavioral effects occurring over time require both neurogenesis-dependent and -independent

processes,<sup>27, 61, 62</sup> such as the progressive desensitization of 5-HT<sub>1A</sub> autoreceptors.<sup>63</sup> As venlafaxine inhibits both NE and 5-HT reuptake, we can speculate that OCT2 deficiency impairs noradrenergic and/or serotonergic signaling in yet undetermined circuits that promote the long-term effects of this antidepressant. Long-term treatment with NRIs and SNRIs including venlafaxine is known to progressively attenuate the firing of NE neurons of the locus coeruleus, possibly via modulation of  $\alpha$ <sub>2</sub>-adrenoceptor function.<sup>62, 64-66</sup> Enhanced NE concentrations in the locus coeruleus or projection regions due to OCT2 deficiency might thus be expected to antagonize the long-term action of venlafaxine. In addition other mechanisms such as the constitutive increase in SERT level or decreased activity of 5-HT<sub>1A</sub> receptors in projection regions, could also contribute to the insensitivity of OCT2 mice to long-term venlafaxine treatment.

In conclusion, our results reveal the role of OCT2 in NE and 5-HT uptake in the brain as a complementary system to the high-affinity transporters, important for mood-related behaviors. They emphasize the importance of OCT2 in the acute action of antidepressants and indicate that this transporter is required for chronic antidepressant treatments to exert their full effects. A striking parallel can be drawn between these observations and the fact that long-term antidepressant treatments that improve symptoms in depressed patients are otherwise inactive on healthy subjects. Since OCT2 appears to have a similar localization in human and rodent brain,<sup>17</sup> this suggests that antidepressant efficacy in man should be amenable to modulation through the manipulation of the function of this transporter.

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### **Conflict of interest**

The authors declare no competing financial interests.

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## Legends to figures

**Figure 1** Specific expression of OCT2 in limbic regions. **(a-i)** Immunofluorescent histochemistry of coronal brain sections from wild-type and OCT2<sup>-/-</sup> mice. High expression of OCT2 is shown in the primary motor (M1), secondary motor (M2) and cingulate (Cg) cortex **(a)**, in CA1 and CA3 of the hippocampus **(b)** and in the amygdala **(c)**. OCT2 is found in some but not all noradrenergic neurons of the locus coeruleus (LC), labeled by tyrosine hydroxylase (TH) antibodies **(d)**, and weakly expressed in the dorsal raphe (DR) in a fraction of serotonergic neurons, labeled by tryptophan hydroxylase (TPH) antibodies **(e)**. **(f-i)** OCT2 is always found in neurons, as shown by co-localization with neuronal marker NeuN in layer V of the secondary motor cortex **(f)**, CA1 of the hippocampus **(g)** and basolateral nucleus of the amygdala (BLA) **(h)** and not in astrocytes, shown with glial fibrillary acidic protein (GFAP) labeling in the cortex **(i)**. Little or no labeling is seen in corresponding sections of OCT2<sup>-/-</sup> mice brain **(a-c)**. Cortical layers are identified by roman numerals. Scale bars represent 100  $\mu\text{m}$  **(b,c)**, 50  $\mu\text{m}$  **(a)** and 20  $\mu\text{m}$  **(d-i)**.

**Figure 2** OCT2<sup>-/-</sup> mice show modifications in anxiety and depression-related behaviors. **(a-c)** OCT2<sup>-/-</sup> mice show decreased inhibition in conflict anxiety paradigms. **(a)** Time and locomotor activity in the center of the open-field are increased in OCT2<sup>-/-</sup> mice (Student's *t*-test, unpaired, two-tailed,  $t_{44} = 3.6$ ,  $***P = 0.0009$  and  $t_{44} = 2.5$ ,  $*P = 0.015$ , respectively,  $n = 16-30$ ). **(b)** Time spent in the open zone of the elevated O-maze is increased in OCT2<sup>-/-</sup> mice (Student's *t*-test, unpaired, two-tailed,  $t_{31} = 2.7$ ,  $**P = 0.01$ ,  $n = 15-18$ ), while latency to enter this zone is reduced ( $t_{31} = 2$ ,  $*P = 0.05$ ,  $n = 15-18$ ). **(c)** Latency to feed in the novelty-suppressed feeding (NSF) test, expressed as fraction of animals that have not eaten over 15 min, is decreased in OCT2<sup>-/-</sup> mice (Mantel-Cox test,  $c_2 = 8$ ,  $df = 1$ ,  $**P = 0.0047$ ,  $n = 9$  per group), while weight loss prior to the test and feeding drive are unaffected. **(d,e)** OCT2<sup>-/-</sup>

mice show increased immobility time in the forced swim test (FST) (Student's *t*-test, unpaired, two-tailed,  $t_{39} = 11.3$ ,  $***P < 0.0001$ ,  $n = 19-22$ ) (d) and the tail suspension test (TST) (Student's *t*-test, unpaired, two-tailed,  $t_{30} = 3$ ,  $**P = 0.0053$ ,  $n = 16$  per group) (e). Results are given as mean  $\pm$  s.e.m..

**Figure 3** OCT2 participates in NE and 5-HT clearance in aminergic projection regions. (a-c) Decrease in uptake, tissular content and clearance of NE and 5-HT in OCT2<sup>-/-</sup> mice. (a) *Ex vivo* NE and 5-HT uptake is decreased in the hippocampus (HPC) and cortex (Cx) of OCT2<sup>-/-</sup> mice. Decynium 22 (D22)-sensitive [<sup>3</sup>H] NE, 5-HT and DA uptake in cell extracts from wild-type and OCT2<sup>-/-</sup> mice brain is expressed as mean  $\pm$  s.e.m. of percentage values of wild-type. Student's *t*-test, paired, two-tailed, NE: HPC,  $t_5 = 3.1$ ,  $*P = 0.028$ ; Cx,  $t_5 = 7.2$ ,  $***P = 0.0008$ ; 5-HT: HPC,  $t_5 = 11.6$ ,  $***P < 0.0001$ ; Cx,  $t_5 = 4.96$ ,  $**P = 0.0042$ ;  $n = 6$ . (b) Decreased intratissular level of NE and 5-HT in OCT2<sup>-/-</sup> mice brain. HPLC results are presented as mean  $\pm$  s.e.m. Student's *t*-test, unpaired, two-tailed, NE: Cortex,  $t_9 = 2.5$ ,  $*P = 0.0338$ ,  $n = 5-6$ ; Striatum,  $t_{10} = 2.6$ ,  $*P = 0.025$ ,  $n = 6$ ; Hippocampus,  $t_{10} = 3.1$ ,  $*P = 0.012$ ,  $n = 6$ ; Brain stem,  $t_9 = 3.5$ ,  $**P = 0.007$ ,  $n = 5-6$ ; Cerebellum,  $t_{10} = 2.5$ ,  $*P = 0.03$ ,  $n = 6$ ; 5-HT: Striatum,  $t_{10} = 2.8$ ,  $*P = 0.019$ ,  $n = 6$ ; Hippocampus,  $t_{10} = 2.4$ ,  $*P = 0.037$ ,  $n = 6$ ; Hypothalamus,  $t_9 = 3.6$ ,  $**P = 0.0055$ ,  $n = 5-6$ . (c) Recovery of pyramidal neuron firing activity *in vivo* after NE and 5-HT application in wild-type and OCT2<sup>-/-</sup> mice. (c, top) Representative integrated firing histograms showing the effects of microiontophoretically-applied NE and 5-HT on the spontaneous activity of CA3 pyramidal neurons, before and following the systemic administration of two cumulative doses of venlafaxine (8 mg per kg). Black and white squares indicate the duration of iontophoretic ejection of NE and 5-HT, respectively. The arrow indicates the time at which the injection of venlafaxine was complete. (c, bottom). Recovery time (RT<sub>50</sub>) values for pyramidal neuronal activity following microiontophoretic applications

of NE and 5-HT are increased in OCT2<sup>-/-</sup> mice after venlafaxine injection. Two-way ANOVA of RT<sub>50</sub> values reveal significant main effects of genotype ( $F_{1,34} = 15.7$ ;  $P = 0.0004$ ), treatment ( $F_{2,34} = 61.3$ ;  $P < 0.0001$ ), and genotype x treatment interaction ( $F_{2,34} = 5.5$ ;  $P = 0.0083$ ) for NE (n = 3–13), as well as significant main effects of genotype ( $F_{1,51} = 32.8$ ;  $P < 0.0001$ ), treatment ( $F_{2,51} = 194.9$ ;  $P < 0.0001$ ), and genotype x treatment interaction ( $F_{2,51} = 10.1$ ,  $P = 0.0002$ ) for 5-HT (n = 4–22). Fisher's post-hoc test reveal significant increases after venlafaxine injection: NE, 16 mg per kg venlafaxine, wild-type mice and OCT2<sup>-/-</sup> mice,  $***P < 0.0001$ ; 8 mg per kg venlafaxine, OCT2<sup>-/-</sup> mice,  $**P = 0.0027$ ; 5-HT, 16 mg per kg venlafaxine, wild-type mice and OCT2<sup>-/-</sup> mice,  $***P < 0.0001$ ; 8 mg per kg venlafaxine, OCT2<sup>-/-</sup> mice,  $***P < 0.0001$ . Significant increases are found in OCT2<sup>-/-</sup> mice compared to wild-type after 8 mg per kg venlafaxine ( $^{\#}P = 0.028$  for NE and  $^{\#\#}P = 0.0035$  for 5-HT) and after 16 mg per kg venlafaxine ( $^{\#\#\#}P = 0.0009$  for NE and  $^{\#\#\#}P < 0.0001$  for 5-HT). **(d)** Diminished clearance in OCT2<sup>-/-</sup> mice cortex and hippocampus is not due to alterations in the expression of other high- or low-affinity transporters. All transporters are found at a comparable or increased level in mutant mice. IL, infralimbic cortex; Cg, cingulate cortex; DG, dentate gyrus. Student's *t*-test, unpaired, two-tailed, SERT: Cg,  $t_9 = 2.6$ ,  $*P = 0.026$ ; CA3,  $t_9 = 4.1$ ,  $**P = 0.0028$ ; DG,  $t_9 = 2.6$ ,  $*P = 0.028$ ; OCT3: CA3,  $t_9 = 2.5$ ,  $*P = 0.034$ ; n = 5–6. Representative sections of specific ligands binding (NET and SERT) or immunohistochemistry (OCT3 and PMAT) are shown on the right. Values are given as mean  $\pm$  s.e.m. **(e-f)** Expression of OCT2 in the body and dendrites of aminergic projection neurons. Immunofluorescent histochemistry of coronal brain sections. OCT2 is distributed in the somato-dendritic compartment, shown by double labeling with microtubule-associated protein 2 (MAP2) antibodies in neurons of layer V of the motor cortex (Cx), hippocampus CA1 and basolateral amygdala nucleus (BLA) **(e)**. OCT2 does not co-localize with the pre-synaptic side of nerve endings labeled with antibodies against the synaptic vesicle protein

synaptophysin, shown in cortex and hippocampus CA3 (f). Scale bars represent 20  $\mu\text{m}$  (e) and 10  $\mu\text{m}$  (f).

**Figure 4** Altered sensitivity to antidepressants in OCT2<sup>-/-</sup> mice in a behavioral despair paradigm. (a-c) Immobility time in the forced swim test (FST) after acute injection of saline or various doses of venlafaxine (a), reboxetine (b) or citalopram (c) in wild-type and mutants. Two-way ANOVA (n = 6–12) reveal significant main effects of genotype ( $F_{1,81} = 8.08$ ;  $P = 0.0056$ ), treatment ( $F_{3,81} = 32.2$ ;  $P < 0.0001$ ), and genotype x treatment interaction ( $F_{3,81} = 7.9$ ;  $P < 0.0001$ ) for venlafaxine; main effects of treatment ( $F_{3,88} = 55.9$ ;  $P < 0.0001$ ) and genotype x treatment interaction ( $F_{3,88} = 17.5$ ;  $P < 0.0001$ ) for reboxetine; main effects of genotype ( $F_{1,77} = 29.2$ ;  $P < 0.0001$ ), treatment ( $F_{3,77} = 24.8$ ;  $P < 0.0001$ ), and genotype x treatment interaction ( $F_{3,77} = 3.1$ ;  $P = 0.03$ ) for citalopram. Fisher's post-hoc test, \*\*\* $P < 0.001$  versus saline; # $P < 0.05$ , ## $P < 0.01$ , ### $P < 0.001$  versus wild-type. (d) Endogenous OCT2 is insensitive to citalopram. D22-sensitive [<sup>3</sup>H] 5-HT uptake in cortex extracts in the presence of increasing concentrations of citalopram (0.1 to 10 mM) is expressed as percentage of D22-sensitive uptake (n = 4). Results are given as mean  $\pm$  s.e.m..

**Figure 5** Impaired reversal of a corticosterone-induced depression-like state by long-term venlafaxine treatment in OCT2<sup>-/-</sup> mice. (a-c). (a) Experimental scheme. The repercussions of a long-term treatment with venlafaxine on depression-like behaviors induced by a chronic corticosterone treatment are evaluated in wild-type and OCT2<sup>-/-</sup> mice. Vertical arrows indicate the time points of behavioral testing. (b) Chronic corticosterone treatment (CORT) alters significantly the behaviors of both wild-type and OCT2<sup>-/-</sup> mice, provoking anhedonia, increasing anxiety, and inducing deterioration of grooming behavior and coat state. Two-way ANOVA (n = 10–14) reveal a significant effect of corticosterone treatment on sucrose

consumption ( $F_{1,37} = 53.9$ ;  $P < 0.0001$ ), time in open zone in elevated O-maze ( $F_{1,42} = 18.1$ ;  $P < 0.0001$ ), grooming frequency in splash test ( $F_{1,41} = 37.8$ ;  $P < 0.0001$ ) and coat state ( $F_{1,50} = 301.1$ ;  $P < 0.0001$ ). Fisher's post-hoc test,  $*P < 0.05$ ,  $**P < 0.01$ ,  $***P < 0.001$  versus untreated mice. Decreased anxiety and increased grooming behavior at basal state are found in  $OCT2^{-/-}$  mice,  $^{\#}P = 0.019$  and  $^{\#}P = 0.044$  versus wild-type mice, respectively. (c)  $OCT2^{-/-}$  mice are insensitive to reversal of corticosterone-induced depression-like behaviors by long-term venlafaxine treatment. Two-way ANOVA ( $n = 6-12$ ) reveal a significant effect of venlafaxine on sucrose consumption ( $F_{1,39} = 5$ ;  $P = 0.032$ ), grooming frequency in splash test ( $F_{1,37} = 5.7$ ;  $P = 0.022$ ) and coat state ( $F_{1,22} = 4.6$ ;  $P = 0.043$ ). Fisher's post-hoc test reveal significant effect of venlafaxine in wild-type mice for sucrose consumption ( $***P = 0.0004$ ), splash test ( $*P = 0.047$ ) and coat state ( $**P = 0.0046$ ), but not in  $OCT2^{-/-}$  mice ( $P = 0.54$ ,  $0.21$  and  $0.82$  for sucrose consumption, splash test and coat state, respectively). Confirming this effect, significant differences are found between venlafaxine-treated wild-type and  $OCT2^{-/-}$  mice in sucrose solution intake ( $^{\#\#}P = 0.0011$ ) and coat state ( $^{\#\#}P = 0.0068$ ). Unpaired two-tailed Student's  $t$ -test revealed significant effects of venlafaxine in elevated O-maze ( $t_{10} = 2.7$ ,  $^{\S}P = 0.021$ ,  $n = 6$ ) in wild-type and not in  $OCT2^{-/-}$  mice ( $t_{11} = 0.2$ ,  $P = 0.83$ ,  $n = 6-7$ ). All values are given as mean  $\pm$  s.e.m..













